Fluorescence Quenching and Inclusion Complex Analysis of Propranolol and β-cyclodextrin

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OBJECTIVES

Propranolol is an amphipathic β-blocker drug used for its regulatory effects over blood pressure and anxiety levels.

Recent studies have explored interactions of propranolol in various environments with respect to its pharmaceutical characteristics; i.e. cellular take-up, intracellular location, etc.

We are interested in expanding the knowledge base of data and analysis on the relation between propranolol and β-, HP-β-, and γ-cyclodextrins, with respect to solvent accessibility.

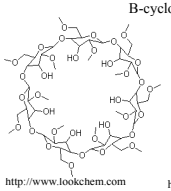
The association constant of propranolol and β-cyclodextrin and the Stern-Volmer quenching relationship with iodide were found by fluorescence spectroscopy.

BACKGROUND

Cyclodextrins are used in a variety of ways, most often in industrial and pharmacoligical applications. They are cyclic oligosaccharides that range from 6 to 100+ units in size; β- and γ-cyclodextrins have 6 and 7 units, respectively. They are capable of inclusion complexes with various molecules, particularly due to their cone-shaped ring structure. Complexation can aid in a number of functions, such as enhancing fluorescence in the presence of a quencher.

B-Cyclodextrin
$C_{42}H_{20}O_{35}$
•MW of 1134.98
• 6 glucopyranose units
•forms inclusion complexes
•Helps prevent quenching of included compounds

B-cyclodextrin



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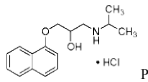
Propranolol hydrochloride is an amphipathic drug capable of interacting with cell membranes. It has a MW of 295.81.



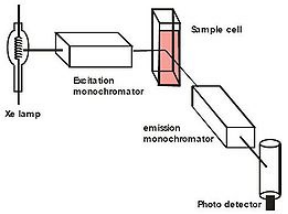
Propranolol HCl

METHODS

Inclusion Complex/Quenching Study – β-cyclodextrin

Stock solutions of propranolol (1.0 x 10^{-3} M), β-cyclodextrin (16 mM), and Potassium Iodide (0.5 M) were created with 0.1M, pH=~7.0 phosphate buffer as the solvent. Exactly 100μL of propranolol, a specified volume of 0.5M KI, and increasing volumes of β-CD were added to five 10mL volumetric flasks and diluted to achieve a final concentration of 10μM propranolol and 0mM, 1.5mM, 3mM, 6mM, and 12mM respective concentrations of β-CD. This was repeated for different specified final concentrations of KI, ranging from 0M to 0.08M. Samples were then excited at 296nm in a Jobin Yvon Horiba FluorMax-3 Fluorimeter, and spectra were measured from 300-450nm.

Fluorometric Analysis



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Fluorescence is the emission lower energy light when excited by high-energy light, usually ultraviolet. Only certain molecules can fluoresce, and each molecule has a unique excitation wavelength and emission wavelength(s). Fluorescence varies based on concentration of the fluorophore and presence of a quencher.

RESULTS

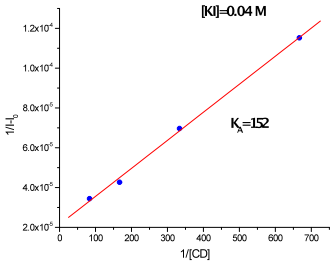
Spectra, Intensity, and Stern-Volmer Plot

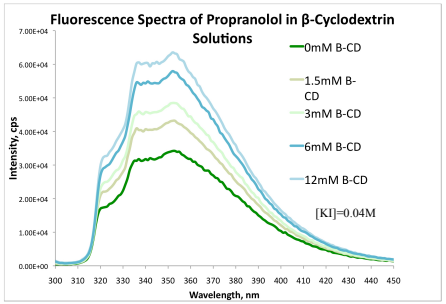
The fluorescence intensity spectra for data collected in one concentration of the quencher shows the fluorescence-enhancing effects of β-Cyclodextrin. In order to find the association constant of β-Cyclodextrin and propranolol in the presence of KI, the inverse of the difference between the intensity (sans iodide or β-CD) and the observed intensity at a given condition. This was done according to the equation below. The Stern-Volmer plot usually gives the association constant and "Stern-Volmer constants" but more data is needed.

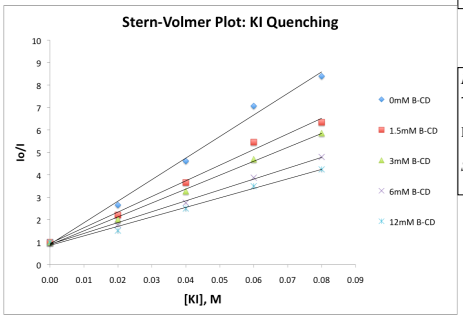
$P + CD \leftrightarrows P - CD$

$$\frac{1}{I - I_0} = \frac{1}{K_A (I_\infty - I_0)} \times \frac{1}{[CD]} + \frac{1}{(I_\infty - I_0)}$$

Association Constant Analysis







CONCLUSIONS

Our observations show that the association constant of β-cyclodextrin and propranolol in the presence of potassium iodide is 152 M^{-1}. Qualitative analysis shows the effects of quenching by potassium iodide, as with many salts. This is seen in the fluorescence spectra at 0.04M KI and the Stern-Volmer plot. The Stern Volmer plot trendlines show the effects of adding increasingly higher concentrations of β-cyclodextrin in order to enhance fluorescence of propranolol by sheltering it from the surrounding salt anions in solution.

The association constant from a previous study was found to be 195 M^{-1} by spectroscopic analysis and 239 M^{-1} by calorimetry.

The results confirm that β-cyclodextrin increases fluorescence under quenching conditions.

FUTURE WORK

As this is an ongoing study, more data will be pursued to increase accuracy of the presented analysis, with respect to β-cyclodextrin. Attempts will be made to analyze the association constants and quenching properties of the compounds in question across a variety of pH, temperature, and cyclodextrin derivatives. At the outset of the study, β-cyclodextrin was to be compared with 2-hydroxypropyl-β-cyclodextrin and γ-cyclodextrin inclusion complexes with propranolol. The study will aim to provide the same types of analysis for these two cyclodextrins. 2-hydroxypropyl-β-cyclodextrin is a synthetically derived version of β-cyclodextrin, with increased solubility characteristics because of its added polar substituent.

After this study is completed, it would be beneficial to gain knowledge of the performance and location of the cyclodextrin derivatives *in vivo*.

REFERENCES

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